Butyrospermol Fatty Acid Esters from the Fruit of a Chinese Mangrove *Xylocarpus granatum*

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A mixture of four new butyrospermol and two known β-sitosterol fatty acid esters, was obtained from the fruit of a Chinese mangrove *Xylocarpus granatum*. The structures of these compounds were established on the basis of spectroscopic data and chemical means. The butyrospermol fatty acid esters were characterized as mainly comprising butyrospermol 3β-*O*-palmitate (**1**), butyrospermol 3β-*O*-oleate (**2**), butyrospermol 3β-*O*-stearate (**3**) and butyrospermol 3β-*O*-linoleate (**4**). The β-sitosterol fatty acid esters were identified as mainly containing β-sitosterol 3β-*O*-myristate and β-sitosterol 3β-*O*-oleate.

Key words: Butyrospermol Fatty Acid Ester, *Xylocarpus granatum*

Introduction

The mangrove *Xylocarpus granatum* is known for producing antifeedant limonoids, especially phragmalins and mexicanolides. Previous investigations on the seeds of two meliaceae plants of mangrove, *X. granatum* and *X. moluccensis*, uncovered an obacunol, two phragmalins, three andirobins and fourteen mexicanolides, including xyloccensins A – K [1 – 5]. Recently, we have reported the isolation and identification of eight unique 8,9,30-phragmalin *ortho* esters and eight new mexicanolides from the bark and fruit of a Chinese mangrove *X. granatum* [6 – 12]. Five new phragmalins [13], among which four were the same as we have reported [9], were obtained from the bark of the same plant. In the current paper, we present the isolation and characterization of a mixture of four new butyrospermol and two known β-sitosterol fatty acid esters from the fruit of *X. granatum*. The structures of these compounds were established on the basis of spectroscopic data and chemical means. The butyrospermol fatty acid esters were characterized as mainly containing butyrospermol 3β-*O*-palmitate (**1**), butyrospermol 3β-*O*-oleate (**2**), butyrospermol 3β-*O*-stearate (**3**) and butyrospermol 3β-*O*-linoleate (**4**). The β-sitosterol fatty acid esters were identified as mainly containing β-sitosterol 3β-*O*-myristate and β-sitosterol 3β-*O*-oleate.

Results and Discussion

A mixture of butyrospermol and β-sitosterol fatty acid esters was isolated from the fruit of *X. granatum* by silica column chromatography. Alkaline hydrolysis of this material with sodium hydroxide yielded two mixtures. One was a mixture of fatty acids, and the other was a mixture of butyrospermol and β-sitosterol. The mixture of fatty acids was methylated with excess trimethylsilyl-diazomethane ($TMSCHN_2$) and characterized by GC-MS to be mainly composed of methyl myristate (3.3%), methyl palmitate (62.2%), methyl oleate (20.1%), methyl stearate (9.8%), and methyl linoleate (4.5%). On the other hand, the second mixture was further purified by silica column chromatography and identified as consisting of butyrospermol [14] and β-sitosterol [15]. The negative ESI-MS spectrum of the whole mixture showed a series of molecular ion $[M-H]^-$ peaks at *m/z* = 623 [β-sitosterol 3β-*O*-myristate $-$ H]$^-$, 663 [butyrospermol 3β-*O*-palmitate $-$ H]$^-$, 677 [β-sitosterol 3β-*O*-oleate $-$ H]$^-$, 687 [butyrospermol 3β-*O*-linoleate $-$ H]$^-$, 689 [butyrospermol 3β-*O*-oleate $-$ H]$^-$, 691 [butyrospermol 3β-*O*-stearate $-$ H]$^-$ and the fragment ions at *m/z* = 227 [myristic acid $-$ H]$^-$, 255 [palmitic acid $-$ H]$^-$, 279 [linoleic acid $-$ H]$^-$, 281 [oleic acid $-$ H]$^-$, 283 [stearic acid $-$ H]$^-$, as well as the positive ESI-MS spectrum of the whole

mixture showed a series of molecular ion peaks at *m/z* = 647 [β-sitosterol 3β-*O*-myristate + Na]$^+$, 663 [β-sitosterol 3β-*O*-myristate + K]$^+$, 665 [butyrospermol 3β-*O*-palmitate + H]$^+$, 682 [butyrospermol 3β-*O*-palmitate + NH_4]$^+$, 689 [butyrospermol 3β-*O*-linoleate + H]$^+$, 693 [butyrospermol 3β-*O*-stearate + H]$^+$, 696 [β-sitosterol 3β-*O*-oleate + NH_4]$^+$, 710 [butyrospermol 3β-*O*-stearate + NH_4]$^+$, 711 [butyrospermol 3β-*O*-linoleate + Na]$^+$, 729 [butyrospermol 3β-*O*-oleate + K]$^+$, 731 [butyrospermol 3β-*O*-stearate + K]$^+$ and the fragment ions at *m/z* = 397 [β-sitosterol − OH]$^+$, 409 [butyrospermol − OH]$^+$. Moreover, the molecular formulae of butyrospermol fatty acid esters **1**–**4** (Chart 1) were determined as $C_{46}H_{80}O_2$, $C_{48}H_{82}O_2$, $C_{48}H_{84}O_2$ and $C_{48}H_{80}O_2$, respectively, by the corresponding HR-ESI-MS data [*m/z* = 663.6077, calcd for [M − H]$^-$ 663.6080 (**1**); 689.6233, calcd for [M − H]$^-$ 689.6237 (**2**); 691.6391, calcd for [M − H]$^-$ 691.6393 (**3**); 687.6076, calcd for [M − H]$^-$ 687.6080 (**4**)]. Based on these results, the initial mixture was characterized as mainly consisting of butyrospermol 3β-*O*-palmitate (**1**), butyrospermol 3β-*O*-oleate (**2**), butyrospermol 3β-*O*-stearate (**3**), butyrospermol 3β-*O*-linoleate (**4**), β-sitosterol 3β-*O*-myristate and β-sitosterol 3β-*O*-oleate (Fig. 1).

Butyrospermol is a protolimonoid. Its fatty acid esters are rare in nature. To date, only the acetate derivative was reported from the latex of *Euphorbia broteri* [14] and the root of *Cudrania javanensis* [16]. To the best of our knowledge, this is the first time to find long chain fatty acid esters of butyrospermol as natural products.

Experimental Section

General experimental procedures

Optical rotations were recorded on a POLAPTRONIC HNQW5 automatic high-resolution polarimeter (Schmidt & Haensch Co. Ltd.). IR spectra were recorded on a Perkin-Elmer FT-IR 1760X spectrophotometer. NMR spectra were recorded in $CDCl_3$ using a Bruker AV-500 spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane as the internal standard. Electrospary ionization (ESI)-MS spectra were measured on a Bruker APEX II spectrometer in positive or negative ion mode. GC-MS was performed with a Daojin QP5050A GC/MS spectrometer employing the EI mode (70 eV). Conditions: DB-17 capillary column (30 m × 0.25 mm × 0.25 μm); column temperature: 150–260 °C, then held at 260 °C; carrier gas: He; injector and detector temperatures: 250 °C and 270 °C, respectively. Preparative HPLC was carried out on ODS columns (250 × 10 mm i. d., YMC) with a Waters 996 photodiode array detector. For CC, silica gel (200–300 mesh) (Qingdao Mar. Chem. Ind. Co. Ltd.) was used.

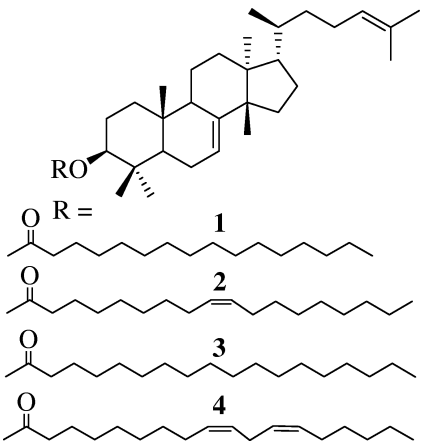

Fig. 1. Structures of compounds **1**–**4**.

Plant material

The fresh fruit of the mangrove *Xylocarpus granatum* was collected in June 2005 from Hainan Island, southern China. The identification of the plant was performed by Prof. Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample (NO. GKLMMM-002-2) is maintained in the Herbarium of the South China Sea Institute of Oceanology.

Extraction and isolation

The dried fruits (8 kg) of *X. granatum* were extracted with hot 95% ethanol three times. The extract was concentrated under reduced pressure, followed by suspension in water. After defatting with *n*-hexane, the aqueous layer was further extracted with ethyl acetate. The ethyl acetate extract (220 g) was chromatographed on silica CC and eluted using a chloroform-methanol system (100 : 0 ∼ 2 : 1) to yield 120 fractions. Fractions 4–10 were combined and further purified with silica CC (petroleum ether-ethyl acetate 30 : 1) to afford a mixture of butyrospermol and β-sitosterol fatty acid esters (1.5 g).

Butyrospermol: colorless crystals. $[\alpha]_D^{25}$ + 15 (*c* 1.0, chloroform). – IR (KBr): $\tilde{\nu}$ = 2940, 1735, 1640, 1470, 1450, 1370, 1250 and 1025 cm^{-1}. – ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 0.74 (s, Me-19), 0.80 (s, Me-18), 0.84 (d, *J* = 5.0 Hz, Me-21), 0.86 (s, Me-30), 0.97 (s, Me-28), 0.97 (s, Me-29), 1.60 (s, Me-26), 1.68 (s, Me-27), 3.25 (dd, *J* = 11.5, 4.1 Hz, H-3), 5.09 (t, *J* = 7.0 Hz, H-24), 5.25 (d, *J* = 3.4 Hz, H-7). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 37.2 (C-1, t), 27.7 (C-2, t), 79.3 (C-3, d), 39.0 (C-4, s), 50.7 (C-5, d), 24.0

(C-6, t), 117.8 (C-7, d), 145.9 (C-8, s), 49.0 (C-9, d), 35.0 (C-10, s), 18.2 (C-11, t), 33.8 (C-12, t), 43.6 (C-13, s), 51.3 (C-14, s), 34.0 (C-15, t), 28.5 (C-16, t), 53.2 (C-17, d), 13.1 (C-18, q), 22.1 (C-19, q), 35.8 (C-20, d), 18.6 (C-21, q), 35.2 (C-22, t), 25.4 (C-23, t), 125.1 (C-24, d), 130.9 (C-25, s), 17.6 (C-26, q), 25.7 (C-27, q), 27.6 (C-28, q), 27.3 (C-29, q), 14.7 (C-30, q). – ESI-MS *m/z* = 425 $[M-H]^-$.

Alkaline hydrolysis: A mixture of butyrospermol and β-sitosterol fatty acid esters (0.5 g) was treated with excess sodium hydroxide (1.5 g) and refluxed in a mixture of dioxane and water (1 : 1, 30 mL) for 16 h. The reaction mixture was concentrated under reduced pressure and extracted with chloroform (3×50 mL) in water (50 mL). The chloroform layer was dried and applied to silica CC (petroleum ether–acetone 10 : 1) to yield butyrospermol and β-sitosterol. After extraction with chloroform three times, the aqueous layer of the reaction mixture was neutralized with hydrochloric acid and concentrated under reduced pressure. The residue was extracted with chloroform three times again to yield a mixture of fatty acids which was treated with excess $TMSCHN_2$ (5 mL) in anhydrous *n*-hexane and stirred at room temperature overnight to afford a colorless wax of fatty acid methyl esters for GC-MS analysis.

GC-MS analysis: Peak 1 (t_R 13.367 min, methyl myristate, 3.3%). – MS (EI, 70 eV) *m/z* = 242 $[M]^+$, 211 $[M-CH_3O]^+$, 185 $[M-CH_2CH_2CH_3]^+$, 157, 143, 129, 101, 87, 74, 55, 43, 29. Peak 2 (t_R 15.158 min, methyl palmitate 62.2%). – MS (EI, 70 eV) *m/z* = 270 $[M]^+$, 239 $[M-CH_3O]^+$, 227 $[M-CH_2CH_2CH_3]^+$, 213, 199, 185, 171, 157, 143, 129, 115, 97, 87, 74, 55, 43, 29. Peak 3 (t_R 16.017 min, methyl linoleate 4.5%). – MS (EI, 70 eV) *m/z* = 294 $[M]^+$, 262 $[M-CH_3OH]^+$, 227, 213, 199, 185, 159, 146, 133, 121, 109, 101, 95, 81, 73, 67, 55, 41. Peak 4 (t_R 16.808 min, methyl oleate 20.1%). – MS (EI, 70 eV) *m/z* = 296 $[M]^+$, 264$[M-CH_3OH]^+$, 222, 180, 137, 123, 110, 98, 87, 74, 69, 55, 43, 41, 29. Peak 5 (t_R 16.933 min, methyl stearate 9.8%). – MS (EI, 70 eV) *m/z* = 298 $[M]^+$, 266 $[M-CH_3OH]^+$, 255 $[M-CH_2CH_2CH_3]^+$, 241, 227, 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 57, 43, 41, 29.

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